

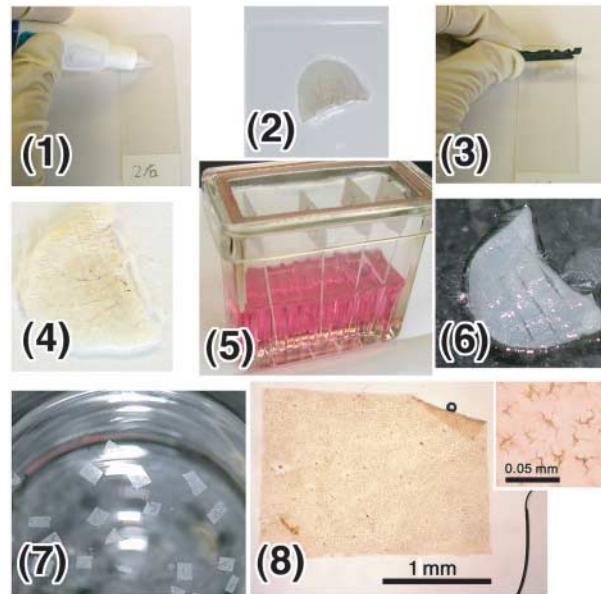


Fig. 13. Our procedure to obtain murine epidermal sheets. (1) Just after a mouse was sacrificed, we cut off both auricles with fine scissors. Immediately, we dropped Bond Aron Alpha High Speed EX[®] (Toagosei Co., Ltd., made of cyano acrylate) on a microslide glass (APS-coated SUPERFROST[®], Matsunami Glass IND., LTD.). (2) Immediately, we glued the auricle and microslide glass, maintaining it as superficial and parallel with the posterior auricle (treated surface). (3), (4) The specimen was shaved gently with a razor blade (Feather-S blade[®]). Next, the specimen was gently cut in checkers pattern (each square had 2-to 3-mm sides). (5) The microslide glasses were immersed in 20 mM Na4-ethylendiamine tetraacetic acid in phosphate-buffered saline, pH 7.4, for 4 h at 37°C. (6) The epidermal sheets were separated from the dermis with fine forceps under a stereoscopic microscope. (7) Obtained epidermal sheets were kept flat by floating them on trisimal buffer (20 mM trisimal maleate, 0.2 M sucrose, pH 7.2) for 30 min. (8) One of the epidermal sheets (after ADPase histochemistry; a modification of the technique described by Chaker *et al.*, 1984).

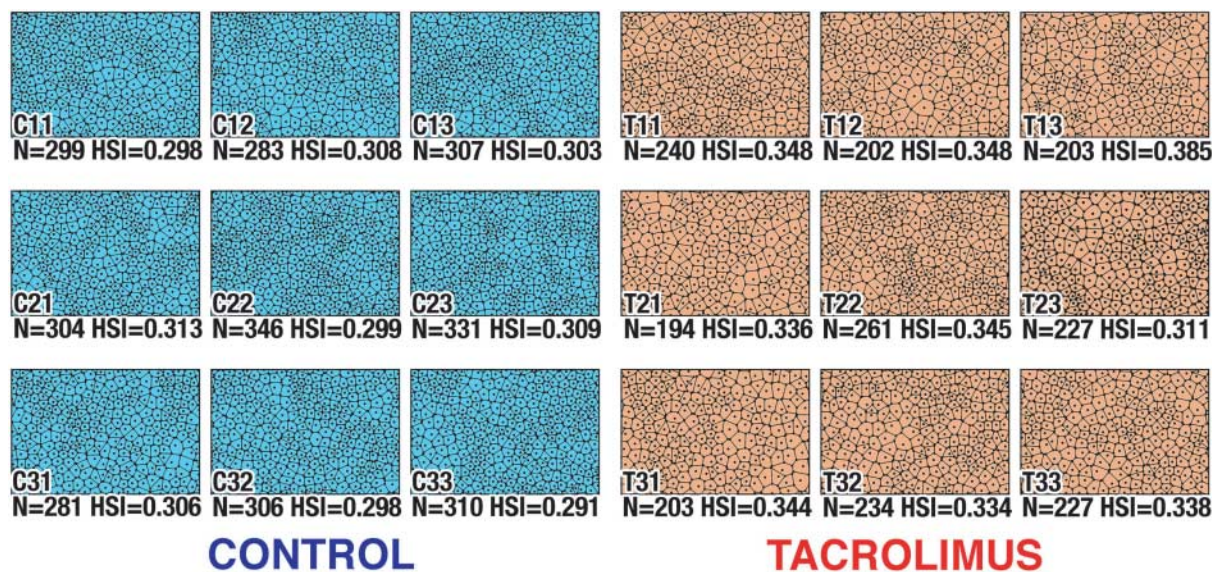


Fig. 14. The Voronoi tessellations for the murine epidermal Langerhans cells. CONTROL: untreated. TACROLIMUS: every posterior auricle was treated with 0.1% tacrolimus ointment (Protopic[®] Ointment 0.1%) once a day for 3 weeks. Each area is a rectangle (600 × 400 μm). N is the number of LCs. HSI is the Hopkins-Skellam index. (The regularity increases as the HSI decreases.)

3.5 Geometrical models of territories

The graph in Fig. 15 shows the three models and the observed data of epidermal Langerhans cells. The lines of CONTROL are near the random sequential packing model. The other models were dismissed with a level of significance of 0.05 by the Chi-square test.

3.6 Statistical tests of densities and spatial regularities (Fig. 16)

Three-week treatment course of Protopic[®] Ointment 0.1% application significantly reduced both the density and regularity ($p = 0.0012$ and $p = 0.0118$ by unpaired t-test; $p = 0.0495$ and $p = 0.0495$ by the Mann-Whitney U test, respectively).